Project No. _____

106 11/22/94   **Book No.** _____   **TITLE** pMc9 / new primers.

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~~✕~~ AAT II ✕ 1 : GCC ACC TGA CGT CTA AGA AAC CA
    non dU   31 mer.   TAT TAT C

✕ AAT II ✕ 2 : GTT TCT TAG ACG TCA GGT GGC ACT TTT
    non dU   29 mer

✕ AAT II ✕ 1 : GCC ACC UGA CGA CUA AGA AAC CAU TAT
    dU   31 mer

✕ AAT II ✕ 2 : GTT TCT UAG ACG UCA GGU GGC ACU
    dU   29 mer.   TTT

<u>Purpose</u>: To try to amplify pMc9 with these new pri

increased the annealing to 58° from 56° (previous exp.

enzyme : Taq alone (1U)   primers : 1 non dU } n
         Taq + Deep vent (1 + .01)   2 dU }
         D.V. (0.5 + 1)
                                                3 dU - cell
done in duplicate.
                                                4 1 dU + 2 no dU
12 Rx / enzyme - con premix made.
                                                5. 2 dU + 1 no d
Deep vent buffer was used thro' out.

Taq : buffer   6U   200 µM dNT
       dNTP   12   200 µg Temp
       Template   2.4   Tube ✕ (1 — 11) 1 µM primer
       enzyme   2.4   2 mM Mg
       H₂O   403.2   cons w. try
                   ____
                   480

40 µl / Rx → added dry primer in 10 µl

Witnessed & Understood by me,   **Date** 11/2/94   **Invented by**

Date 11/23/94

Recorded by A. Sitaraman

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primers old ⎫ = 20x   ~~25~~ 10 + 10 µl g each primer al'
old ⎬   (100 µM)
                          ↓
                  180 µl H₂0

primers new ⎫ = ⎫ equivalent amount g each
new ⎬   ⎬
other 2 different combo ⎬ 50 + 50 µl   (16 µM each)
1 dv + other non dv ⎬

DV : buyer   60   DV alone   0.5 µ   r 0
dNTP   12
Temp   2.4   buffer   60   60
enzyme 12.0   (1·.01/x) dNTP   12   12
H₂0   398.6   Temp   2.4   2.4
                    20/x enzyme   2 0   6.0
      480.0   H₂0   402.6   399.6

ube ✕   (12 — 22)   (23 - 33)   (33 - 44)

11, 22, 33 & 44 w/o any primers.

cycling   94°, 3'

    30(94°, 30", 50°, 30', 72°, 2') → 4° soak

samples thrown out   12/19/94

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Taq:
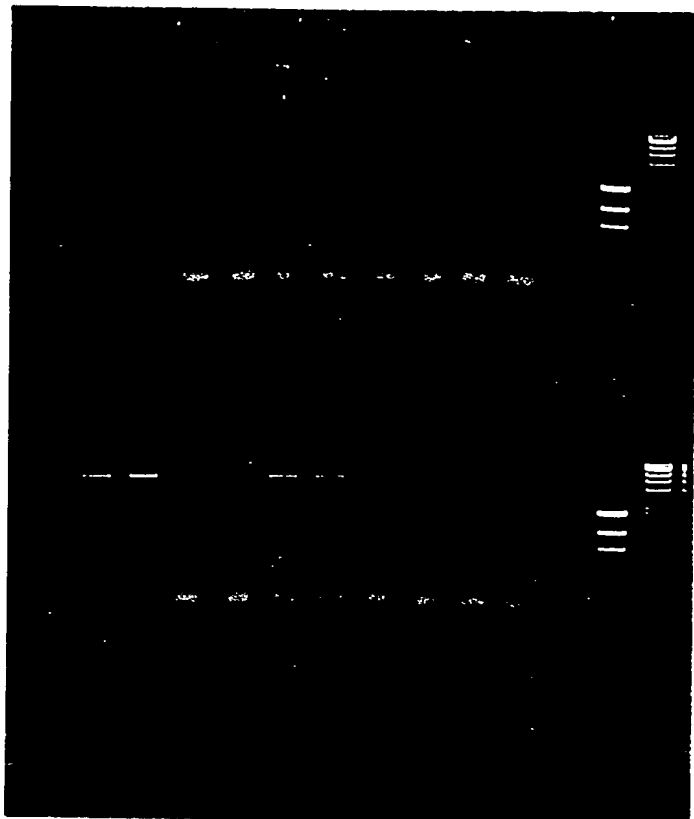
N +   N +   O +   −/+   ~/+   no primer

Taq + D.V   1 : 0.01 :
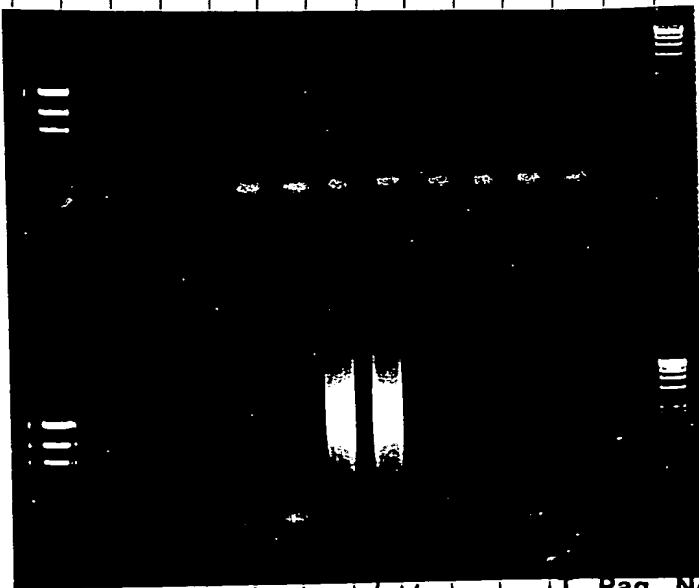
- Deep Vent as usual didn't work ⟶

- with old dv primers smears for the first time.

- Why more primer dimer with dv than with non dv?

Results:

- Even with Taq problems with product at the annealing temp.

- F/R - no dv product se slightly with Taq, more with T + D.V

- old primers at 58°, did work with Taq.

- with Taq + D.V mispri almost gone with old primers new mispri products with T + DV

- mix + match didn't work at all.

D.V   0.50

Witnessed & Understood by me,   Date 11/2/94   Inv nted by   Dat

R c rded by   4/23/94

K. 1. K.......